Population Density as a Predictor of Genetic Variation for Woody Plant Species

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Abstract: *As the focus of conservation biology shifts toward multispecies and ecosystem conservation and management, a principal question becomes how we manage species to conserve their long-term evolutionary potential. Few criteria exist for prioritizing which populations within a species should be protected to conserve maximal genetic variation. We designed this study to explore the genetic consequences of using population density as a criterion for selecting populations of woody plant species for conservation. Population density may be an effective gauge of genetic variation for two reasons. First, density often reflects ecological population size, particularly for continuously distributed species, and density is much easier to measure in the field than population size. Second, from an individual species' perspective, population density may be an indicator of habitat quality. We evaluated the relationship between standard genetic diversity indices and densities of seedlings, small trees, and large trees, and we investigated the association between genotypic composition and density measures with canonical correlation analysis for three common tree species* (Carya tomentosa, Sassafras albidum, *and* Quercus alba) *from the Missouri Ozarks. We found that population density was not correlated with genetic diversity in large populations of plant species, but density was associated with genotypic composition of populations. That is, populations with small densities had different genotypes than those with large densities. To sample a maximal amount of regional genotypic variation, we recommend choosing plant populations representing a range of densities. Findings from our study should be generally applicable to plant populations that have occupied habitats long enough for natural selection to affect local genotypic composition. Used in conjunction with other established criteria, population density may be a useful rule of thumb for conservation practitioners concerned with the maintenance of adaptive genetic variation in plant species.*

Densidad Poblacional como Predictor de la Variación Genética de Especies de Plantas Leñosas

Resumen: *A la vez que el enfoque de la biología de la conservación se sesga hacia la conservación y manejo a nivel multi-especie y de ecosistema, la pregunta: cómo manejar especies para conservar su potencial evolutivo de largo plazo? se convierte en una pregunta importante. Muchas decisiones de conservación son hechas sin datos sobre distribución de la variación genética dentro de las especies. Mas aún, pocos criterios existen para priorizar que poblaciones dentro de especies deberían ser protegidas para conservar la máxima variación genética de la especie. Diseñamos este estudio para explorar las consecuencias genéticas de utilizar la densidad poblacional como un criterio para seleccionar poblaciones de especies de plantas leñosas para su conservación. La densidad poblacional puede ser un estimador efectivo de la variación genéntica por dos razones. Primero, la densidad frecuentemente refleja el tamaño poblacional ecológico, particularmente para especies distribuídas continuamente y la densidad es mucho mas fácil de medir en el campo que el tamaño poblacional. Segundo, desde la perspectiva de especie individual, la densidad poblacional puede ser un indicador de calidad del hábitat. Evaluamos la relación entre índices de diversidad genética típicos y medidas de diversidad en base a un análisis de correlación canónica para tres especies comúnes de árboles* (Carya tomentosa, Sassafras albidum *y* Quercus alba) *del Ozarks de Missouri. Encontramos que la densidad poblacional no estuvo correlacionada con la diversidad genética en poblaciones grandes de especies de pantas, pero*

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la densidad estuvo asociada con la composición genotípica de las poblaciones. Esto es, que las poblaciones con pequeñas densidades tuvieron diferentes genotipos al ser comparadas con poblaciones con densidades grandes. Para muestrear una cantidad máxima de variación genotíon genotípica regional, recomendamos que se seleccionen plantas representantes de un amplio rango de densidades. Los resultados de este estudio podrían ser generalmente aplicables para poblaciones de plantas que ocupan hábitats lo suficientemente grandes como para que la selección satural afecte la composición genotípica local. La densidad poblacional, utilizada en conjunto con otros criterios establecidos, puede ser una regla útil para los conservacionistas que se preocupan por el mantenimiento de la variación adaptativa en especies de plantas.

Introduction

As the focus of conservation biology shifts toward multispecies and ecosystem conservation and management, the emphasis of conservation efforts becomes maintenance of ecosystem integrity (e.g., Likens 1992; Grumbine 1994; Pickett et al. 1997). In contrast to single-species conservation, which attempts to prevent extinction, multispecies or ecosystem-level approaches try to avert species from becoming rare or endangered (Holsinger & Vitt 1997). The principal question becomes how we manage species to conserve their long-term evolutionary potential (Riggs 1990; Falk & Holsinger 1991; Hedrick & Miller 1992; Lande 1995; Avise 1996).

The ability of an organism to adapt to a changing environment depends on the amount of genetic variation present in the species; more genetic variation translates to greater potential for long-term persistence (Frankham 1995). Genetic variation has two separate components, genetic diversity and genotypic composition. Genetic diversity represents the assortment and frequency of alleles at sampled loci. Genetic diversity is quantified with parameters such as percent polymorphic loci, average number of alleles per locus, or expected heterozygosity in a population (Hartl 1988). These indices are similar to parameters in the ecological literature such as species richness and species diversity. Genotypic composition describes how multilocus genotypes are distributed among populations of a species across different habitats or a geographical range (Petit et al. 1998), just as species inventories depict which species are found in which forests.

Genetic markers, such as isozymes (Hamrick & Godt 1996) and microsatellites (Chase et al. 1996; Petit et al. 1998), can be used to describe the amount of genetic variation within and among populations, to distinguish genetically diverse or depauperate populations, and to identify genetically unique populations. Practically, however, genetic information is usually not available for multispecies or habitat conservation plans (Hamrick & Godt 1996), and decisions are based on distribution and abundance data that are traditionally collected in species monitoring programs (e.g., Margules et al. 1988; Scott et al. 1993; Noss & Cooperrider 1994). Ecological factors such as population size and geographical range have been used to predict broad patterns of genetic variation in different plant species (Ellstrand & Elam 1993; Hamrick & Godt 1996), but few ecological criteria exist for prioritizing which populations within a species should be protected to conserve maximal genetic variation (Petit et al. 1998).

We explore the genetic consequences of using population density as a criterion for selecting populations of woody plant species for conservation. Population density may be an effective gauge of genetic variation for two reasons. First, density often reflects ecological population size, particularly for continuously distributed species, and density is much easier to measure in the field than population size. Large populations that can maintain themselves ecologically are not as vulnerable to loss of genetic diversity as are small populations (Lande 1988), suggesting that dense populations may contain more genetic diversity than sparse populations. If genetic diversity is associated with population density, then density may be a reliable ecological indicator of population genetic diversity.

Second, from an individual species' perspective, population density may be an indicator of habitat quality. When density reflects the suitability of environmental conditions for a given population, natural selection may differentially influence the genotypic composition of populations with different densities. For example, if population density varies among microhabitats, selection might favor different genotypes in these different microhabitats, producing an association between genotypic composition and population density. If genotypes are variable among populations, a critical component of preserving maximal genetic variation of a species will be sampling from populations that harbor different genotypes.

Management is the rule rather than the exception as ecosystems around the globe are rapidly degraded (Noss & Cooperrider 1994). Conservation practitioners need practical tools or rules of thumb to implement conservation goals that include the maintenance of adaptive genetic variation. We propose that population density may be a useful criterion for selecting populations for conservation because density is an ecological parameter that may indicate genetic variation. Thus, we explored the relationship between population density and both components of genetic variation in three common woody plant species in Missouri Ozark forests. Specifically, we evaluated the relationship between standard genetic di-

versity indices and densities of seedlings, small trees, and large trees, and we investigated the association between genotypic composition and density measures.

Methods

Study Area

The study area is part of the Missouri Ozark Forest Ecosystem Project (MOFEP), a multi-investigator ecosystem project administered by the Missouri Department of Conservation (Brookshire & Shifley 1997). The MOFEP study area incorporates one wildlife area and four state forests located in the Ozark Mountains of southcentral Missouri: Deer Run State Forest (Reynolds County), Paint Rock, Cardavera, and Carr Creek State Forests (Shannon County), and Peck Ranch Wildlife Area (Carter County). Before 1880 these forests were dominated by continuous *Pinus echinata* (short leaf pine) communities, but intensive harvesting (1880-1920) followed by repeated burning and grazing altered the landscape to produce the mature upland oak-hickory and oak-pine communities found there today (Cunningham & Hauser 1989). In the Ozarks, *Quercus alba* shares the canopy with other species of oaks, including *Q. stellata*, *Q. velutina*, *Q. coccinea*, and with *P. echinata* and *Carya tomentosa* (Kurzejeski et al. 1993).

The study area is divided into nine sites, ranging in size from 260 to 527 ha (Fig. 1). The sites are contiguous tracts of forest with minimal edge, largely free from manipulation for at least 40 years. All the sites are located on land owned by the Missouri Department of Conservation and are in close proximity to one another (Brookshire & Hauser 1993). The MOFEP study area covers 13 microhabitats (i.e., ecological land types, Miller 1981); south- and west-facing slopes, north- and east-facing slopes, and ridge-top microhabitats make up 90% of the total study area (Meinert et al. 1997). Each site is further divided into approximately 5-ha forestry stands within a microhabitat.

To minimize broad environmental differences among populations, we sampled stands from south- and west-facing and north- and east-facing slopes only. Sampled populations ranged in elevation from 182 to 275 m and were between lat 37°00′N and 37°15′N and between long 91°07′W and 91°00′W (U.S. Topographic Maps, 7.5-minute series: Fremont, Van Buren North, Stegall Mountain, Powder Mill Ferry, and Exchange, Missouri). Distances between sampled populations ranged from 0.2–24 km.

Study Species

We selected two canopy trees and one understory shrub to be the focal study species: *Carya tomentosa* Nuttell (Juglandaceae; mockernut hickory); *Quercus alba* L. (Fagaceae; white oak); and *Sassafras albidum* (Nuttell) Nees (Lauraceae). Hereafter, these species will be referred to as *Carya*, *Q. alba*, and *Sassafras*, respectively. We chose these three species because they are widely distributed among MOFEP study sites (Brookshire et al. 1997) and throughout the region (Braun 1950), which makes them ideal representatives of temperate-forest woody plant species. In addition, to rigorously test for associations between density and genetic variables, we needed to sample many populations per species, which would not be possible with an uncommon or sparsely distributed species. Hamrick and colleagues (Loveless & Hamrick 1984; Hamrick et al. 1992) have shown that the life-history traits of a species can influence the distribution of genetic variation, so we sampled species with different life-history characteristics. These species differ in pollen vector, seed-dispersal vector, successional status, canopy status, and abundance in the MOFEP sites (Fig. 2). *Q. alba* is one of the most common canopy tree species, *Carya* occurs at lower densities, and *Sassafras* is one of the more abundant understory trees (Brookshire et al. 1997; Kabrick et al. 1997).

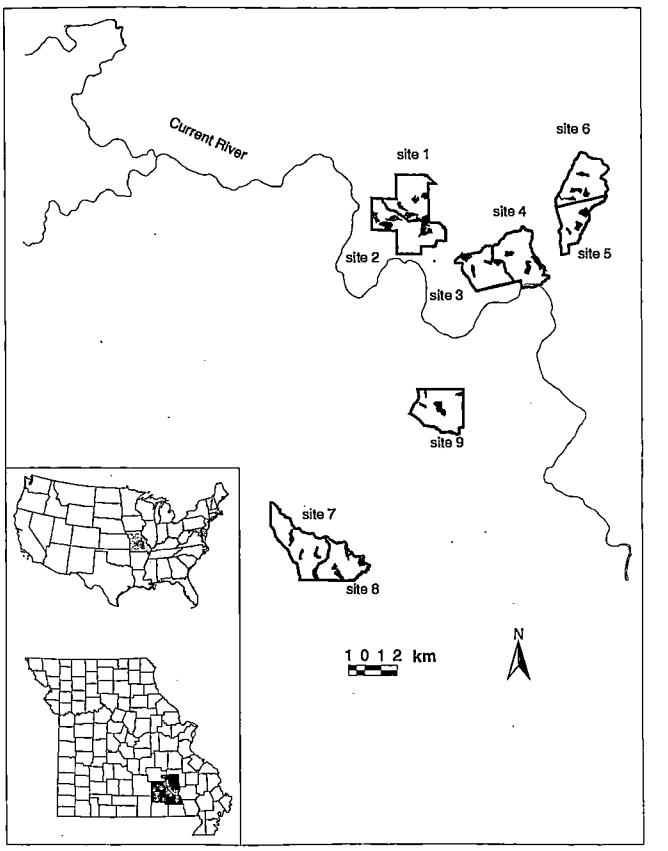

Figure 1. Map of the Missouri Ozark Forest Ecosystem Project study area with study sites 1–9. Forest stands where populations of Carya tomentosa, Sassafras albidum, *and* Quercus alba *were sampled are shaded in black.*

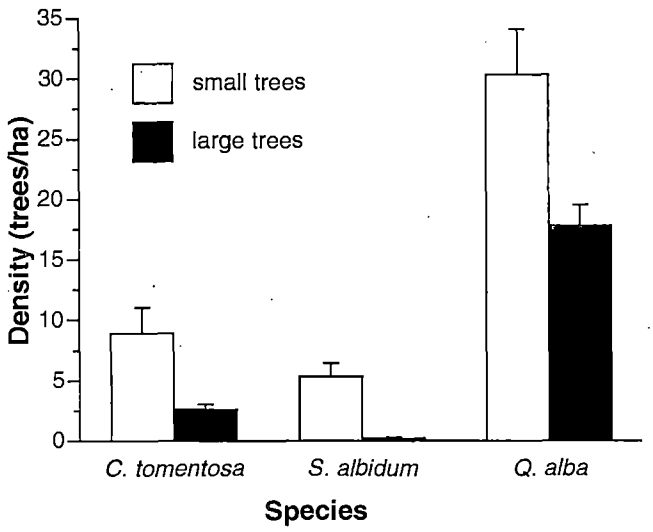

Figure 2. Mean population density (+ 1 SE) of small (4–11 cm diameter at breast height [dbh]) and large (dbh > 11 cm) trees for the three study species (n = *36 populations for each species).*

Carya flowers in Missouri during April and May (Steyermark 1963). Plants are monoecious, with male flowers borne in catkins that are up to 12 cm long and female flowers that occur in dense, short spikes. Pollen is wind-dispersed, and squirrels and gravity are responsible for seed movement. The species is tetraploid (Stone 1961). The second species, *Q. alba*, also flowers in Missouri in April and May (Steyermark 1963). Plants are monoecious, with male flowers borne in catkins and female flowers that are either sessile or on short stalks. The flowers are wind-pollinated, and seeds are dispersed by gravity, mammals, and birds. The third species, *Sassafras*, is usually a shrub or small tree in the Ozarks, even though it can be a canopy species elsewhere. Although this species occurs earlier in succession than the other two species, it can be found throughout MOFEP study sites. The plants are generally dioecious, with the insect-pollinated flowers blooming in April and May. Fruiting is largely confined to forest gaps and edges in this shade-intolerant species. The seeds are dispersed by birds. The genetic structure of the three study species has been described in detail elsewhere (Koop 1996). In general, all three species show significant population differentiation for multilocus genotypes.

Genetic Sampling

The data for this study were collected as part of a genetic survey of tree populations in the MOFEP study area (for full details of sampling and electrophoretic protocols, see Sork et al. 1997). Our goal was to sample 48 individuals from four stands in each of the nine sites of the MOFEP study area, resulting in a total sample size of 36 populations per species. Stands sampled were located on either north and east or south and east slope microhabitats, as identified by Miller (1981). Individual trees were selected from an area of approximately 1 ha within the central part of the microhabitat. As leaves were collected, they were placed on ice and later stored in an ultracold freezer (−70° C).

All leaf samples were analyzed with standard horizontal starch-gel electrophoresis procedures (Kephart 1990). Mature leaf tissue was ground into a fine powder in a mortar by means of liquid nitrogen and a pestle. Protein from each sample was extracted with a phosphate extraction buffer (Mitton et al. 1979), which for *Q. alba* was modified with 10% polyvinyl-pyrrolidone to inactivate phenolics that tend to bind proteins (Kephart 1990). For each species, we surveyed 20 enzymes on several combinations of five gel-electrode buffer systems (Koop 1996; Sork et al. 1997). For the genetic diversity analyses, we selected loci that showed some polymorphism because these loci will reveal the most information about variation across populations (Table 1). For the genetic composition analysis, we used only loci that expressed well across all or most populations (Table 1). We were unable to obtain complete sample sizes for all three species due to a freezer breakdown that allowed degradation of some of the *Carya* samples and to problems with uneven enzyme expression across *Sassafras* individuals—a problem typical of species in the Lauraceae (V.L.S., personal observation). The final sample sizes for statistical analysis were 1077 multilocus genotypes across 10 isozymes for *Carya*; 1717 multilocus genotypes across 10 isozymes for *Q. alba*, and 1094 multilocus genotypes across 5 isozymes for *Sassafras*. *Carya* was the only species with <36 populations represented (n = 32).

Data Analysis

The Missouri Department of Conservation provided density data for each species (collected in 1995), including small and large tree densities and relative abundance of seedlings (Grabner et al. 1997; Kabrick et al. 1997). We calculated separate density measures for each size class in each population to maintain the independent contribution of the three size classes in the multivariate analyses. Densities of small trees (diameter at breast height [dbh] between 4 and 11 cm) and large trees (dbh >11 cm) were obtained from permanent 0.2-ha plots located within each tree stand where genetic populations were sampled. We used percent cover data that were collected in 16, 1-m^2 quadrants located in each permanent 0.2-ha plot to approximate the mean relative abundance of seedlings of each species.

We calculated seven standard measures of genetic diversity for each population: percent polymorphic loci with a maximum allelic frequency of 95% and with no

Table 1. List of isozymes used to compare genetic diversity among populations of three species of woody plants sampled in Missouri Ozark forests.

Carya tomentosa	Quercus alba	Sassafras albidum
Amino acid transferase-2 (Aat-2)*	Colormetric esterase (Ces)*	Amino acid transferase-2 (Aat-2)*
Diaphorase-2 (Dia-2)*	Flourescent esterase (Fes-1)*	Amino acid transferase-3 (Aat-3)*
Fluorescent esterase-1 (Fes-1)	Flourescent esterase (Fes-4)*	Diaphorase-2 (Dia-1)*
Malate dehydrogenase-1 (Mdh-1)	Malate dehydrogenase (Mdh-1)	Diaphorase-2 (Dia-2)*
Malate dehydrogenase-2 (Mdh-2)	Menadione reductase (Mnr-1)*	Menadione reductase-1 (Mnr-2)*
Menadione reductase-1 (Mnr-1)*	Peroxidase (Per-1)*	
Menadione reductase-2 (Mnr-2)	Peroxidase (Per-3)*	
Phosphoglucoisomerase-1 (Pgi-1)	Phosphoglucoisomerase (Pgi-1)	
Phosphoglucoisomerase-2 (Pgi-2)*	Phosphoglucoisomerase (Pgi-2)*	
Shikimate dehydrogenase-2 (Skdh-1)*	Shikimate dehydrogenase-2 (Skdh-1)	

**Polymorphic loci used for the multilocus analysis of genotypic composition of populations.*

frequency criteria (P_{95} and P_{NC}); effective number of alleles and average number of alleles per polymorphic locus (A_e and A_P); observed and expected heterozygosity (H_O and H_E); and population fixation index, or inbreeding coefficient (F_{IS}), which represents the deficiency of heterozygotes in a population (Hartl 1988; Hamrick & Godt 1989).

For analyses of genotypic composition, we used the multilocus genotype of each individual in a population. These genotypes were based on transformations of all diploid and tetraploid genotypes into linear combinations of traits by scoring of each polyploid genotype into a score for each allele minus one (Westfall & Conkle 1992). This multivariate genotypic score was then used for analyses with genotypic composition.

To test for associations between genetic diversity and population density, we performed canonical correlation analyses for each study species. Canonical correlation analysis is a generalized multiple regression between two groups of variables. In this case, we modeled genetic diversity as the dependent variable group and population density as the independent variable group. Initially, we included all seven genetic diversity measures with all three density variables (relative abundance of seedlings, small-tree density, and large-tree density) in the canonical correlation model. To potentially improve the fit of the model, we eliminated variables that had both a low standardized canonical coefficient and a small correlation with their canonical variable in the original model analysis (Westfall & Conkle 1992). We reanalyzed the data with this reduced model and compared results with those of the original model; we kept the model that best fit the data as determined by the overall canonical correlations and the canonical structure (the correlations between each variable and the final significant canonical variables). Residuals from the final models were checked for normality.

We also used a canonical correlation method to test for associations between genotypic composition and population density in each study species. We modeled genotypic composition (i.e., the multilocus genotype of each individual per population) as the dependent variable group and population density as the independent variable group. Following the same procedure described above, we found the best-fit model for each species. Because we used genotypic composition data for each individual tree sampled in a population and population-level density data, the squared canonical correlation (R^2) value obtained in the analysis was devalued. To correct for the lower R^2 values generated when one value (density) is repeated with many different values of the other variable (genotype) in a correlation, we also report the squared Pearson correlation (r^2) between the density canonical variable and mean genotypic composition canonical variable for each population (R. Westfall, personal communication).

To test for the presence of spatial autocorrelation in genetic and density data, we compared geographical distances between populations with genetic and density differences between populations with a series of Mantel tests (Mantel 1967). We used a permutation test with 999 iterations to test for significance. Using Proc Candisc (SAS Institute 1996), we calculated Mahalanobis genetic distances (Mahalanobis 1948) between populations to construct the genetic distance matrices. To construct the density-distance matrix, we calculated Euclidean distances between density measures of each population. Mahalanobis distance was appropriate as a genetic distance measure because it is sensitive to differences among populations due to genetic drift, in contrast to Nei's genetic distance measure, which is sensitive to differences due to mutation (Weir 1990). Because these Ozark populations are relatively young (1–2 generations), population differences are unlikely the result of mutation.

Results

The first hypothesis, that populations with greater density have higher genetic diversity, was not supported. The canonical correlation analysis between genetic di-

versity and population density was not significant for *Carya* ($R^2 = 0.26, p = 0.97$), *Sassafras* ($R^2 = 0.35, p = 0.31$), or *Q. alba* ($R^2 = 0.17, p = 0.94$).

The second hypothesis, that genotypic composition is associated with density, was supported. We found significant canonical correlations between genotypic composition and population density for all three species (Table 2). For *Carya,* the first canonical vector was dominated by the relationship between small-tree density and Pgi-2^2 and Pgi-2^6 alleles; the second canonical vector had relatively high correlations with large tree density and Pgi-2^4, Skdh-1, Skdh-2, and Skdh-3 alleles (Fig. 3a & 3b). For *Sassafras,* the first canonical vector was correlated predominantly with small trees and Mnr-7 allele; the second canonical vector was dominated by seedlings and Dia-2^5, Aat-2^5, and Dia-1^2 alleles (Fig. 3c & 3d). *Q. alba* had only one significant canonical vector, which was correlated mostly with large trees and Fes-4^3 and Fes-4^4 alleles (Fig. 3e).

The Mantel tests for spatial autocorrelation showed a weak but significant correlation between geographical location and density measures ($r^2 = 0.12, p = 0.05$) for *Sassafras* populations but no significant correlations for the other species. We also found no evidence for spatial autocorrelation of genetic measures for any species. Thus, geographic proximity did not explain the distribution of population density or genetic variation in this study.

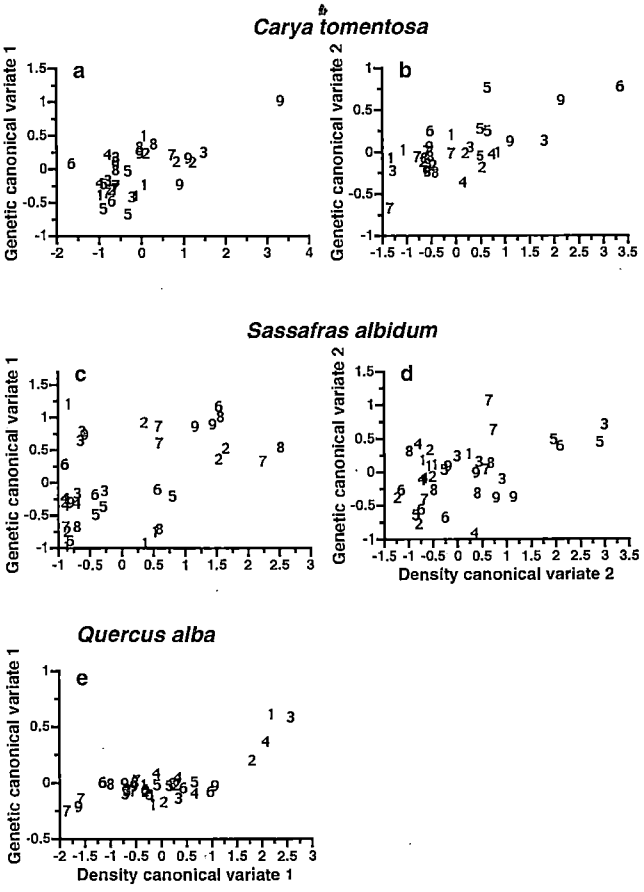

Figure 3. Plots of the canonical variables with significant canonical correlations for Carya tomentosa *(a, b),* Sassafras albidum *(c, d), and* Quercus alba *(e). Populations (*n = *32 for* Carya, n = *36 for* Sassafras *and* Q. alba*) are represented by their study site numbers (1–9).*

Discussion

Density and Genetic Variation

In spite of the expectation that large populations should have greater genetic diversity than smaller populations (Ellstrand & Elam 1993; Frankham 1995), our findings suggest that population density, even when it reflects population size, may not be a good predictor of genetic diversity in woody plant species. Several explanations might account for the lack of association in our data. First, all of our populations may have been sufficiently large to maintain genetic diversity. Low levels of population genetic diversity occur when alleles are lost through genetic drift in small populations or with low levels of gene flow in isolated populations. In contrast, genetic diversity within long-lived woody plant populations is usually high (Hamrick & Godt 1989) because gene flow is sufficient to maintain comparable allelic diversity. Relatively sparse populations of *Carya*, *Sassafras*, and *Q. alba*, all long-lived woody plant species, in the Ozarks may not be small enough to exhibit low genetic diversity measures, precluding patterns of genetic diversity that correlate with species density. To detect a relationship between genetic diversity and population density, we may have needed samples from both very large and very

Table 2. Significant squared canonical correlations (R^2), squared Pearson correlations (r^2) between density and mean genetic composition canonical variables for each population, Wilks' λ, approximate *F*-value, degrees of freedom for numerator and denominator, and the probability of *F* from canonical correlation analysis between genetic composition and population density for *Carya tomentosa, Sassafras albidum,* and *Quercus alba.*

R^2	r^2	*Wilks' λ*	F	*df*	p > F
Carya tomentosa					
0.05[a]	0.38	0.89	2.04	60, 3145	0.0001
0.03[b]	0.37	0.94	1.58	38, 2110	0.01
Sassafras albidum					
0.09[a]	0.21	0.88	7.30	20, 2164	0.0001
0.04[b]	0.20	0.96	4.87	9, 1083	0.0001
Quercus alba					
0.02[a]	0.61	0.97	2.10	21, 4655	0.002

[a]*First significant canonical correlation.*
[b]*Second significant canonical correlation, uncorrelated with first canonical variables.*

small populations. This range of sizes (and correlated densities) may not have occurred in our study area.

A second explanation for the lack of a diversity-density relationship is that local density for a species may not reflect genetic effective population size. Effective population size, N_e, defined as the size of a randomly mating population that results in a given variance in allele frequency or amount of inbreeding (Wright 1931), is a function of the number of founders, the population size of recent generations, and the size of the neighborhood area as a function of gene dispersal distances (Templeton & Read 1994; Hedrick & Gilpin 1997). For populations that are not at equilibrium (a likely scenario for this young forest), density may not reflect effective population size. Direct estimates of effective population size are difficult to measure and are rarely done in habitat conservation plans, discouraging further investigation of the relationship between population density and effective population size. Populations of the three study species were ecologically large, however, suggesting large effective populations as well, and we suspect that the lack of a relationship between density and genetic diversity in our study is primarily a function of indistinguishably high genetic diversity for all sample populations.

The significant relationship between density and genotypic composition for *Carya*, *Q. alba*, and *Sassafras* was, to some extent, surprising. These three species have different habitat preferences, densities, and successional status. That we observed a significant relationship for all three species suggests that a gradient exists between density and the genotypic composition of populations. Most forest stands in the study area are relatively young (<75 years old), suggesting that random founder events outweigh any selection pressures that may have occurred. The data indicate that, for all three species, dense populations may have one set of genotypes, sparse populations may have another set, and intermediate-density populations may display more variable genotypes.

These results could be the outcome of three possible evolutionary scenarios. First, the extremely heterogeneous environment found in the Ozark forests may have quickly selected for specific genotypes. Second, the populations that we find today may have been established by remnants of older populations whose genotypes were already differentiated by selection over many years. Third, founder events for these species may have produced populations with different genotypes. This last scenario seems the least likely. Although founder events could account for differentiation among populations, we would not expect to find a relationship between genotypic composition and density without selection acting as well. We cannot distinguish among the possible processes that created the observed patterns, but our findings demonstrate that selection in some manner must have played a role in shaping the current distribution of genotypes in these species.

As the practice of conservation embraces multispecies and ecosystem management plans, we need to develop tools that guide us in the maintenance of adaptive genetic variation. Results from this study indicate that incorporation of genetic considerations in conservation plans is feasible by means of ecological parameters that can be measured in the field. To the extent that density reflects habitat quality, we suggest that density is a reliable criterion to identify populations with different genotypes for many plant species, except perhaps for species that are sparsely distributed or exist only in isolated populations. Density may also reflect genetic diversity in species with population sizes smaller than those reported in this study. Future studies are needed to investigate these relationships for species with other life-history traits and distribution patterns. Findings from our study should be generally applicable to plant populations that have occupied habitats long enough for natural selection to affect local genotypic composition. To manage species for conservation of their long-term evolutionary potential in the midst of rapidly dwindling native habitat, we must continue to develop practical criteria for assessing and preserving genetic variation.

Implications for Conservation

The three study species exhibited similar relationships between population density and components of genetic variation, suggesting that density may be a functional tool for selecting populations for conservation. We recommend choosing plant populations that represent a range of densities to ensure that a maximal amount of regional genotypic variation is sampled. Preserving populations with different densities increases the likelihood of maintaining a variety of genotypes that will be successful under different environmental conditions. In addition, if all selected populations have sufficiently large population sizes, populations with different densities will not lack genetic diversity. Thus, population density, used in conjunction with other established criteria, may be a useful rule of thumb for conservation practitioners concerned with the maintenance of adaptive genetic variation in plant species.

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Literature Cited

Avise, J. C. 1996. Introduction: the scope of conservation genetics. Pages 1-9 in J. C. Avise and J. L. Hamrick, editors. Conservation genetics: case studies from nature. Chapman & Hall, New York.

Braun, L. 1950. Deciduous forests of eastern North America. McGraw-Hill, New York.

Brookshire, B., and C. Hauser. 1993. The Missouri Forest Ecosystem Project. Pages 289-306 in A. R. Gillespie, G. R. Parker, P. E. Pope, and G. Rink, editors. Proceedings of the ninth Central Hardwood Forest Conference. General technical report NC-161. U.S. Forest Service, North Central Forest Experiment Station, St. Paul, Minnesota.

Brookshire, B. L., and S. R. Shifley, editors. 1997. Proceedings of the Missouri Ozark Forest Ecosystem Project symposium: an experimental approach to landscape research. General technical report NC-193. U.S. Forest Service, North Central Forest Experiment Station, St. Paul, Minnesota.

Brookshire, B. L., R. Jensen, and D. C. Dey. 1997. The Missouri Ozark Forest Ecosystem Project: past, present and future. Pages 1-25 in B. L. Brookshire and S. R. Shifley, editors. Proceedings of Missouri Ozark Forest Ecosystem Project symposium: an experimental approach to landscape research. General technical report NC-193. U.S. Forest Service, North Central Forest Experiment Station, St. Paul, Minnesota.

Chase, M., R. Kesseli, and K. Bawa. 1996. Microsatellite markers from population and conservation genetics of tropical trees. American Journal of Botany **83**:51-57.

Cunningham, R. J., and C. Hauser. 1989. The decline of the Missouri Ozark forest between 1880 and 1920. Pages 34-37 in T. A. Waldrup, editor. Proceedings of pine hardwood mixtures: a symposium on management and ecology of the type. General technical report 58. U.S. Forest Service, Southeast Forest Experiment Station, Atlanta.

Ellstrand, N. C., and D. R. Elam. 1993. Population genetics of small population size: implications for plant conservation. Annual Review of Ecology and Systematics **23**:217-242.

Falk, D. A., and K. E. Holsinger, editors. 1991. Genetics and conservation of rare plants. Oxford University Press, New York.

Frankham, R. 1995. Conservation genetics. Annual Review of Genetics **29**:305-327.

Grabner, J. K., D. R. Larsen, and J. M. Kabrick. 1997. An analysis of MOFEP ground flora: pre-treatment conditions. Pages 169-197 in B. L. Brookshire and S. R. Shifley, editors. Proceedings of Missouri Ozark Forest Ecosystem Project symposium: an experimental approach to landscape research. General technical report NC-193. U.S. Forest Service, North Central Forest Experiment Station, St. Paul, Minnesota.

Grumbine, R. E. 1994. What is ecosystem management? Conservation Biology **8**:27-38.

Hamrick, J. L., and M. J. W. Godt. 1989. Allozyme diversity in plant species. Pages 43-63 in A. H. D. Brown, M. T. Clegg, A. L. Kahler, and B. S. Weir, editors. Plant population genetics, breeding and genetic resources. Sinauer Associates, Sunderland, Massachusetts.

Hamrick, J. L., and M. J. W. Godt. 1996. Conservation genetics of endemic plant species. Pages 281-303 in J. C. Avise and J. L. Hamrick, editors. Conservation genetics. Chapman and Hall, New York.

Hamrick, J. L., M. J. W. Godt, and S. L. Sherman-Broyles. 1992. Factors influencing levels of genetic diversity in woody plant species. New Forests **6**:95-124.

Hartl, D. L. 1988. A primer of population biology. Sinauer Associates, Sunderland, Massachusetts.

Hedrick, P. W., and M. E. Gilpin. 1997. Genetic effective size of a metapopulation. Pages 166-181 in I. Hanski and M. E. Gilpin, editors. Metapopulation biology: ecology, genetics, and evolution. Academic Press, San Diego.

Hedrick, P. W., and P. S. Miller. 1992. Conservation genetics: techniques and fundamentals. Ecological Applications 2:30-46.

Holsinger, K. E., and P. Vitt. 1997. The future of conservation biology: what's a geneticist to do? Pages 202-216 in S. T. A. Pickett, R. S. Ostfeld, M. Shachak, and G. E. Likens, editors. The ecological basis of conservation. Chapman and Hall, New York.

Kabrick, J. M., D. R. Larsen, and S. R. Shifley. 1997. Analysis of pretreatment MOFEP woody vegetation and environmental data: evaluation for compartment, block, and treatment trends in species counts, density, diameter and basal area. Pages 150-168 in B. L. Brookshire and S. R. Shifley, editors. Proceedings of Missouri Ozark Forest Ecosystem Project symposium: an experimental approach to landscape research. General technical report NC-193. U.S. Forest Service, North Central Forest Experiment Station, St. Paul, Minnesota.

Kephart, S. R. 1990. Starch gel electrophoresis of plant isozymes: a comparative analysis of techniques. American Journal of Botany 77:693-712.

Koop, A. L. 1996. Genetic variation and structure in *Quercus alba* L. in a Missouri Ozark landscape. Master's thesis. University of Missouri, St. Louis.

Kurzejeski, E. W., R. L. Clawson, R. B. Renken, S. L. Sheriff, L. D. Vangilder, C. Hauser, and J. Faaborg. 1993. Experimental evaluation of forest management: the Missouri Ozark Forest Ecosystem Project. Transactions of the North American Wildlife and Natural Resource Conference **58**:599-609.

Lande, R. 1988. Genetics and demography in biological conservation. Science **241**:1455-1460.

Lande, R. 1995. Breeding plans for small populations, based on the dynamics of quantitative genetic variance. Pages 318-340 in J. D. Ballou, M. Gilpin, and T. J. Foose, editors. Population management for survival and recovery. Columbia University Press, New York.

Likens, G. E. 1992. The ecosystem approach: its use and abuse. Ecology Institute, Oldendorf/Luhe, Germany.

Loveless, M. D., and J. L. Hamrick. 1984. Ecological determinants of genetic structure in plant populations. Annual Review of Ecology and Systematics **15**:65-95.

Mahalanobis, P. C. 1948. Historic note on the D^2 statistic. Sankhya 9:237.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research **27**:209-220.

Margules, C. R., A. O. Nicholls, and R. L. Pressey. 1988. Selecting networks of reserves to maximise biological diversity. Biological Conservation **43**:63-76.

Meinert, D., T. Nigh, and J. Kabrick. 1997. Landforms, geology and soils of the MOFEP study sites. Pages 56-68 in B. L. Brookshire and S. R. Shifley, editors. Proceedings of Missouri Ozark Forest Ecosystem Project symposium: an experimental approach to landscape research. General technical report NC-193. U.S. Forest Service, North Central Forest Experiment Station, St. Paul, Minnesota.

Miller, M. R. 1981. Ecological land classification terrestrial subsystem: a basic inventory system for planning and management of the Mark Twain National Forest. U.S. Forest Service, Rolla, Missouri.

Mitton, J. B., Y. B. Linhart, K. B. Sturgeon, and J. L. Hamrick. 1979. Al-

lozyme polymorphism detected in mature needle tissue of ponderosa pine. Journal of Heredity 70:86–89.

Noss, R. F., and A. Y. Cooperrider. 1994. Saving nature's legacy: protecting and restoring biodiversity. Island Press, Washington, D.C.

Petit, R. J., A. E. Mousadik, and O. Pons. 1998. Identifying populations for conservation on the basis of genetic markers. Conservation Biology 12:844–855.

Pickett, S. T. A., R. S. Ostfeld, M. Shachak, and G. E. Likens, editors. 1997. The ecological basis of conservation: heterogeneity, ecosystems, and biodiversity. Chapman and Hall, New York.

Riggs, L. A. 1990. Conserving genetic resources on-site in forest ecosystems. Forest Ecology and Management 35:45–68.

SAS Institute. 1996. SAS. Version 6.12. SAS Institute, Cary, North Carolina.

Scott, J. M., F. Davis, B. Csuti, R. Noss, B. Butterfield, C. Groves, H. Anderson, S. Caicco, F. D'Erchia, T. C. Edwards Jr., J. Ulliman, and R. G. Wright. 1993. Gap analysis: a geographic approach to protection of biological diversity. Wildlife Monographs 123.

Sork, V. L., A. Koop, M. A. de la Fuente, P. Foster, and J. Raveill. 1997. Patterns of genetic variation in woody plant species in the Missouri Ozark Forest Ecosystem Project (MOFEP). Pages 231–247 in B. L. Brookshire and S. R. Shifley, editors. Proceedings of Missouri Ozark Forest Ecosystem Project symposium: an experimental approach to landscape research. General technical report NC-193. U.S. Forest Service, North Central Forest Experiment Station, St. Paul, Minnesota.

Steyermark, J. A. 1963. Flora of Missouri. The Iowa State University Press, Ames.

Stone, D. E. 1961. Ploidal level and stomatal size in the American hickories. Brittonia 13:293–302.

Templeton, A. R., and B. Read. 1994. Inbreeding: one word, several meanings, much confusion. Pages 91–106 in V. Loeschcke, J. Tomiuk, and S. K. Jain, editors. Conservation genetics. Birkhäuser Verlag, Basel, Switzerland.

Weir, B. S. 1990. Genetic data analysis: methods for discrete population genetic data. Sinauer Associates, Sunderland, Massachusetts.

Westfall, R. D., and M. T. Conkle. 1992. Allozyme markers in breeding zone designation. New Forest 6:279–309.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 16:97–159.